

## Dehalogenation of the Herbicides Bromoxynil (3,5-Dibromo-4-Hydroxybenzonitrile) and Ioxynil (3,5-Diiodo-4-Hydroxybenzonitrile) by *Desulfitobacterium chlororespirans*

Alison M. Cupples,<sup>1\*</sup> Robert A. Sanford,<sup>2</sup> and Gerald K. Sims<sup>1</sup>

United States Department of Agriculture—Agricultural Research Service,<sup>1</sup>  
and Department of Geology, University of Illinois at  
Urbana—Champaign,<sup>2</sup> Urbana, Illinois

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*Desulfitobacterium chlororespirans* has been shown to grow by coupling the oxidation of lactate to the metabolic reductive dehalogenation of *ortho* chlorines on polysubstituted phenols. Here, we examine the ability of *D. chlororespirans* to debrominate and deiodinate the polysubstituted herbicides bromoxynil (3,5-dibromo-4-hydroxybenzonitrile), ioxynil (3,5-diiodo-4-hydroxybenzonitrile), and the bromoxynil metabolite 3,5-dibromo-4-hydroxybenzoate (DBHB). Stoichiometric debromination of bromoxynil to 4-cyanophenol and DBHB to 4-hydroxybenzoate occurred. Further, bromoxynil (35 to 75  $\mu$ M) and DBHB (250 to 260  $\mu$ M) were used as electron acceptors for growth. Doubling times for growth (means  $\pm$  standard deviations for triplicate cultures) on bromoxynil ( $18.4 \pm 5.2$  h) and DBHB ( $11.9 \pm 1.4$  h), determined by rate of [ $^{14}$ C]lactate uptake into biomass, were similar to those previously reported for this microorganism during growth on pyruvate (15.4 h). In contrast, ioxynil was not deiodinated when added alone or when added with bromoxynil; however, ioxynil dehalogenation, with stoichiometric conversion to 4-cyanophenol, was observed when the culture was amended with 3-chloro-4-hydroxybenzoate (a previously reported electron acceptor). To our knowledge, this is the first direct report of deiodination by a bacterium in the *Desulfitobacterium* genus and the first report of an anaerobic pure culture with the ability to transform bromoxynil or ioxynil. This research provides valuable insights into the substrate range of *D. chlororespirans*.

An increasingly popular approach for the remediation of halogenated environmental pollutants is biological reductive dehalogenation. Dechlorination is the most commonly reported reductive dehalogenation process, due to the widespread pollution of chlorinated compounds (e.g., tetrachloroethene, trichloroethene, or chlorinated phenols), with few reports on the reduction of other halogens. However, the expanding list of emerging halogenated contaminants, including polybrominated fire retardants (20), disinfection by-products (32), perfluorinated surfactants (5), and pesticides (9, 26), illustrates the need for a greater understanding of defluorination, debromination, and deiodination. To address this, the unexploited potential of previously isolated chlororespiring microorganisms is of particular interest.

Bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) is used for the postemergence control of annual broad-leaved weeds in cereals, maize, sorghum, flax, allium species, mint, and grass seed crops (35) (Fig. 1). The octanoate ester of bromoxynil (3,5-dibromo-4-cyanophenyl octanoate), also an herbicide, is readily degraded in nonsterile water and in other biological systems to bromoxynil (35). Ioxynil (3,5-diiodo-4-hydroxybenzonitrile) (Fig. 1) is used for the postemergence control of annual broad-leaved weeds in cereals, onions, leeks, shallots, flax, sugar cane, forage grasses, lawns, and turf (35).

The need for a comprehensive understanding of bromoxynil degradation is indicated by the toxicity associated with bromoxynil (classified as a group C possible human carcinogen and considered to be developmentally toxic [46]), along with reports of its off-site movement in wind-eroded sediment (23) and in waters (17, 28, 31) associated with agricultural areas. Detectable levels of bromoxynil in blood plasma were found in 19.3% of the rural residents tested in Saskatchewan, Canada, even though bromoxynil application in the region had not occurred for 5 months (40). Identifying the dissipation mechanisms would clearly be advantageous for elucidating bromoxynil's fate in the environment and, consequently, the risk of human exposure.

Under aerobic conditions, both in the soil and in enrichment and pure cultures, bromoxynil degradation products frequently retain the bromine groups. The fate of ioxynil is less obvious, as few degradation studies have been reported. However, one could hypothesize that ioxynil is subject to a similar pathway (also retaining the halogens) because of the structural similarity of these two herbicides. The most commonly reported aerobic bromoxynil transformation products are 3,5-dibromo-4-hydroxybenzamide and 3,5-dibromo-4-hydroxybenzoate (DBHB) (15, 16, 27, 37, 42, 43, 47, 48). DBHB can be formed directly from the parent (27) or via 5-dibromo-4-hydroxybenzamide (15, 43, 48). The common occurrence of brominated metabolites indicates that a more efficient strategy for complete bromoxynil removal should include debromination.

Understanding dissipation pathways under anaerobic condi-

\* Corresponding author. Mailing address: United States Department of Agriculture—Agricultural Research Service, S-306 Turner Hall, 1102 S. Goodwin Ave., Urbana, IL 61801. Phone: (650) 244-6096. Fax: (217) 333-5251. E-mail: acupples@uiuc.edu.

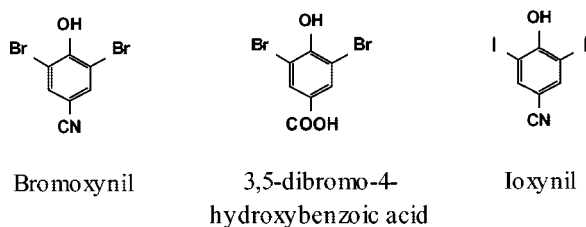


FIG. 1. Chemical structures of compounds investigated.

tions is important because agricultural regions frequently contain anaerobic areas. Direct evidence for anaerobic conditions in soil was elegantly presented using oxygen microelectrodes to measure oxygen profiles within soil aggregates (41). It has also been reported that areas of major herbicide use in North Central states have soils that are predisposed to flooding due to seasonal rainfall and poor drainage (8). Thus, it is of general interest to understand how herbicides such as bromoxynil are metabolized under anaerobic conditions. The soil microbial isolate *Desulfitobacterium chlororespirans* uses *ortho* substitution chlorophenols and chlorohydroxybenzoates as electron acceptors for growth under anaerobic conditions (38). Here, we investigate the ability of this organism to dehalogenate bromoxynil, ioxynil, and the bromoxynil metabolite DBHB (Fig. 1). This paper describes the susceptibility of the brominated compounds to anaerobic metabolic reductive dehalogenation and the cometabolic deiodination of ioxynil by *D. chlororespirans*.

#### MATERIALS AND METHODS

**Chemicals.** Bromoxynil (99%) and ioxynil (99%) were purchased from Chem Service (West Chester, PA), and 3,5-dibromo-4-hydroxybenzoate (99%) (DBHB), 4-cyanophenol (95%), 4-hydroxybenzoate (99%), 3-chloro-4-hydroxybenzoate (CIOHB), and lactate (60% wt/wt syrup) were obtained from Sigma-Aldrich (St. Louis, MO).

**Culture and growth conditions.** All cultures were grown with 50 ml of medium (as described below) in 120-ml serum bottles with butyl rubber stoppers crimped on with aluminum seals. The mineral and trace metal medium recipes were as previously described (38). To this, 1 ml vitamin stock solution (50) and resazurin (20 mg liter<sup>-1</sup>) were added. Following this, the medium was purged with N<sub>2</sub>:CO<sub>2</sub> (80:20) for 0.5 h and moved to an anaerobic chamber (90% N<sub>2</sub>, 5% H<sub>2</sub>, and 5% CO<sub>2</sub>). Sodium sulfide (1.5 μM) was added, the medium was distributed to serum bottles, and the bottles were then autoclaved.

**Chemical analysis.** Bromoxynil, ioxynil, DBHB, 4-cyanophenol, 4-hydroxybenzoate, and CIOHB were quantified by reverse-phase high-pressure liquid chromatography (HPLC) (series 1050; Hewlett Packard, San Fernando, CA). Conditions were as follows: injector volume, 50 μl; mobile-phase flow rate, 0.7 ml min<sup>-1</sup>; UV detector wavelength, 218 nm (bromoxynil, DBHB, 4-hydroxybenzoate, and CIOHB), 235 nm (ioxynil), or 250 nm (4-cyanophenol); reverse-phase C<sub>18</sub> column (ProntoSIL C18-EPS); and an isocratic mobile phase (acetonitrile: water:acetic acid, 49.75:49.75:0.5).

Periodically, 400- to 500-μl samples were removed and filtered (polytetrafluoroethylene filter, 0.22 μm, 4 mm; Alltech Associates Inc., Deerfield, IL) from each bottle for HPLC analysis. The samples were analyzed either immediately or later; if analyzed later, 5 μl of NaOH (2 M) was added and the samples were stored (4°C) until analysis.

**Batch studies.** Inocula of *Desulfitobacterium chlororespirans* used for batch studies were grown with media (as described above), CIOHB (1 mM), and lactate (1 mM). In all experiments, any liquid additions to batch bottles were filter sterilized (polyethersulfone filter, 0.2 μm, 25 mm; Millipore, Co. Cork, Ireland). All batch bottles were incubated (37°C) in the dark on a rotary shaker.

**DBHB experiment.** On day 0, lactate (1 mM), DBHB (5 μM), and 1 ml cells were added to triplicate bottles. The control consisted of the same, except without cells. On day 7, DBHB (70 μM) was added to all four bottles. On days 9 and 10, both DBHB (110 μM and 430 μM) and lactate (1 mM) were added to

all four bottles. Finally, on day 11, DBHB (570 μM) and lactate (1 mM) were added to each bottle (except control).

**Growth studies.** To test for growth on bromoxynil, on day 0, triplicate bottles received bromoxynil (35 to 75 μM), lactate (1 mM), 1 ml cells, and 90 μl [U-<sup>14</sup>C]lactate (0.1 μCi μl<sup>-1</sup>) (American Radiolabeled Chemicals, Inc., St. Louis, MO). On day 4 or 5 and on day 6, bromoxynil (70 to 82 μM and 94 μM, respectively) was added to the culture bottles. To test for growth on DBHB, triplicate bottles received DBHB (250 to 260 μM), lactate (1 mM), 1 ml cells, and 50 μl [U-<sup>14</sup>C]lactate (0.1 μCi μl<sup>-1</sup>). Both experiments included two controls (no cells or no electron acceptor). The final specific activities in the media were 2.20 × 10<sup>8</sup> dpm (100 μCi) and 3.98 × 10<sup>8</sup> dpm (181 μCi) per mmol lactate for experiments with DBHB and bromoxynil, respectively. Samples were removed periodically for HPLC analysis and analysis of <sup>14</sup>C incorporation into biomass, as previously described (21). Briefly, samples were filtered, and the radioactivity associated with the filters (indicating <sup>14</sup>C incorporation into biomass) was determined by scintillation counting.

To obtain cell concentration (mg liter<sup>-1</sup>) from disintegrations per minute per milliliter, the empirical formula for biomass of C<sub>5</sub>H<sub>7</sub>O<sub>2</sub>N was used (34). Based on the balance of electron equivalents, the stoichiometry for cell synthesis was 0.6 mmol of cell mass per mmol lactate assimilated when lactate was used as the electron donor and carbon source (34): CH<sub>3</sub>CHOHCOO<sup>-</sup> + 0.4 H<sup>+</sup> + 0.6 NH<sub>4</sub><sup>+</sup> → 0.6 C<sub>5</sub>H<sub>7</sub>O<sub>2</sub>N + 1.8 H<sub>2</sub>O. Cell synthesis during metabolic reductive dehalogenation of DBHB was determined as follows:

$$\text{cell concentration} \left( \frac{\text{mg}}{\text{liter}} \right) = \frac{{}^{14}\text{C assimilated} \left( \frac{\text{dpm}}{\text{ml}} \right) \times \left( \frac{0.6 \text{ mmol of cells}}{\text{mmol of lactate}} \right) \times \left( \frac{113 \text{ mg of cells}}{\text{mmol of cells}} \right)}{\left( \frac{\text{liter}}{1,000 \text{ ml}} \right) \times \left( \frac{2.2 \times 10^8 \text{ dpm}^*}{\text{mmol of lactate}} \right)}$$

For the reductive dehalogenation of bromoxynil, 3.98 × 10<sup>8</sup> dpm per mmol lactate was used (\*).

Doubling times of *D. chlororespirans* during reductive dehalogenation of the brominated compounds were determined from the increase in <sup>14</sup>C-labeled cell mass during log-phase growth.

**Ioxynil experiment.** On day 0, lactate (1 mM), ioxynil (12 μM), and 1 ml cells were added to triplicate bottles. Two sets of triplicate bottles received the same contents; to one set bromoxynil (40 μM) was added, and to the other CIOHB (1 mM) (a previously reported electron acceptor [38]) was added. An uninoculated control was included for each treatment. Additionally, at day 36, more ioxynil (55 μM) was added to the ioxynil-only treatment bottles, CIOHB (1 mM) was added to the bromoxynil-ioxynil treatment bottles, and as a positive control, CIOHB was added to one of the ioxynil-CIOHB treatment bottles.

**GC-MS analyses.** Metabolites were identified using gas chromatography-mass spectrometry (GC-MS) by comparison of retention times and mass spectra with those of standard compounds. Results were confirmed by comparison to library spectra (National Institute of Standards and Technology mass spectral search program, version 2.0a). Electron ionization mass spectra were obtained using an Agilent 6890N gas chromatograph coupled to an Agilent 5973 mass-selective detector. The injector was operated in splitless mode at a temperature of 250°C, and the system was operated under constant flow conditions (1 ml min<sup>-1</sup>). Helium was the carrier gas, and the column was a model HP-5MS column (30-m by 0.25-mm inside diameter, 0.25-μm film thickness). The temperature program was as follows: 85°C for 5 min, followed by a temperature ramp of 20°C min<sup>-1</sup> up to 300°C, with the final temperature being held for 5 min. The electron ionization conditions were as follows: ionization energy, 70 eV; electron multiplier voltage, 1,047 V; filament emission current, 35 μA; ionization temperature, 230°C; mass range *m/z*, 35 to 550; and scan time, 0.35 s. The sample volume injected ranged from 1 to 2 μl. Samples were subjected to solid-phase extraction (Alltech vacuum manifold; Extract-Clean C<sub>18</sub> 200-mg columns) prior to GC-MS analyses. Solid-phase extraction columns were conditioned with 1 ml methanol, and then a 10- to 20-ml sample was loaded onto the column; finally, the sample was eluted with 1 ml acetonitrile.

#### RESULTS

**DBHB debromination.** DBHB was depleted repeatedly in triplicate culture samples (Fig. 2A) with conversion to 4-hydroxybenzoate (Fig. 2B), whereas no depletion of DBHB or formation of 4-hydroxybenzoate was observed in the medium

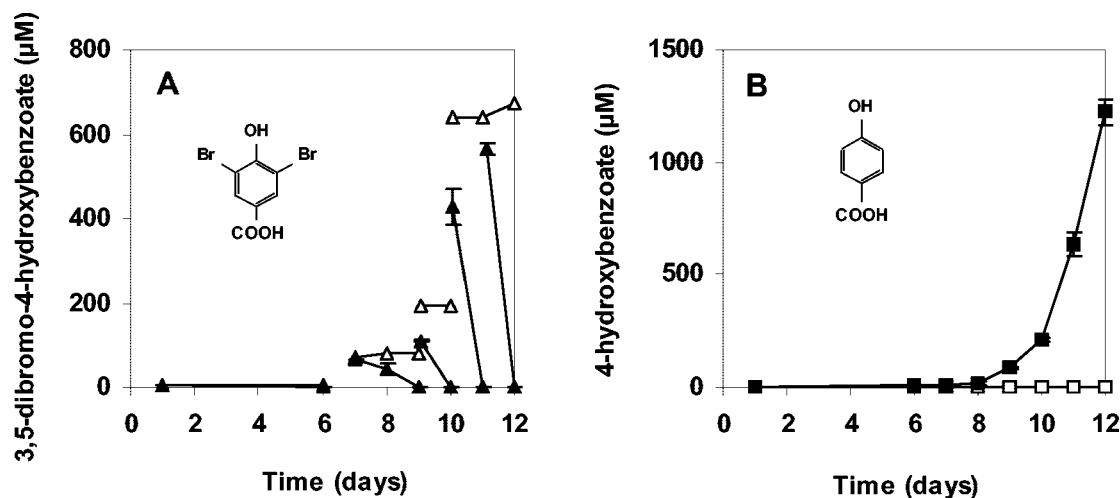


FIG. 2. DBHB in triplicate cultures (filled triangles) and medium control (open triangles) (samples were initially supplied with 4.6  $\mu\text{M}$  DBHB) (A) and corresponding 4-hydroxybenzoate (debrominated product) in cultures (filled squares) and medium control (open squares) (B). Points and error bars represent means and standard deviations for triplicate cultures.

control (Fig. 2). The debrominated product (4-hydroxybenzoate) was identified from mass spectra (data not shown). No 4-hydroxybenzoate was observed in the ion chromatogram of the control (data not shown).

**Microorganism growth.** We investigated cell growth during DBHB and bromoxynil debromination by measuring  $^{14}\text{C}$  uptake in samples provided with  $[^{14}\text{C}]$ lactate (Fig. 3 and 4). As noted previously during growth via metabolic reductive dehalogenation by *Anaeromyxobacter dehalogenans* with  $[^{14}\text{C}]$ acetate (21), the detectability of  $[^{14}\text{C}]$  assimilation was extremely useful for the accurate observation of small incremental increases in biomass in cultures with no visible cell yields. DBHB debromination to 4-hydroxybenzoate (Fig. 3A) coincided with biomass increase, with no  $[^{14}\text{C}]$ lactate uptake if either cells or DBHB was omitted (Fig. 3B). These data illustrate that *D. chlororespirans* can use DBHB as an electron acceptor for growth (doubling time was equal to  $11.9 \pm 1.4$  h [mean  $\pm$  standard deviation for triplicate cultures]), obtaining energy through metabolic reductive dehalogenation.

Bromoxynil was repeatedly depleted in triplicate culture samples (Fig. 4A) with conversion to 4-cyanophenol (Fig. 4B), whereas no depletion of bromoxynil or formation of 4-cyanophenol was observed in the medium control (Fig. 4A and B). The debrominated product, 4-cyanophenol, was identified from mass spectra (data not shown). No 4-cyanophenol was observed in the ion chromatogram of the control (data not shown). Debromination of bromoxynil to 4-cyanophenol coincided with biomass increase, with no  $[^{14}\text{C}]$ lactate uptake if either cells or bromoxynil was omitted (Fig. 4C). These data indicate that *D. chlororespirans* can also use bromoxynil as an electron acceptor for growth. The doubling time during growth on bromoxynil was  $18.4 \pm 5.2$  h (mean  $\pm$  standard deviation for triplicate cultures). The debromination activity on both bromoxynil and DBHB was transferable (transferred three times to fresh medium containing the brominated compound as the only electron acceptor), providing additional evidence that metabolic reductive dehalogenation was occurring. Further confirmation of growth via metabolic reductive dehaloge-

nation was provided by the determination of  $f_e$  (the fraction of electrons from the electron donor being used to reduce bromoxynil or DBHB) and  $f_s$  (the fraction of electrons from the electron donor being incorporated into the biomass). Using

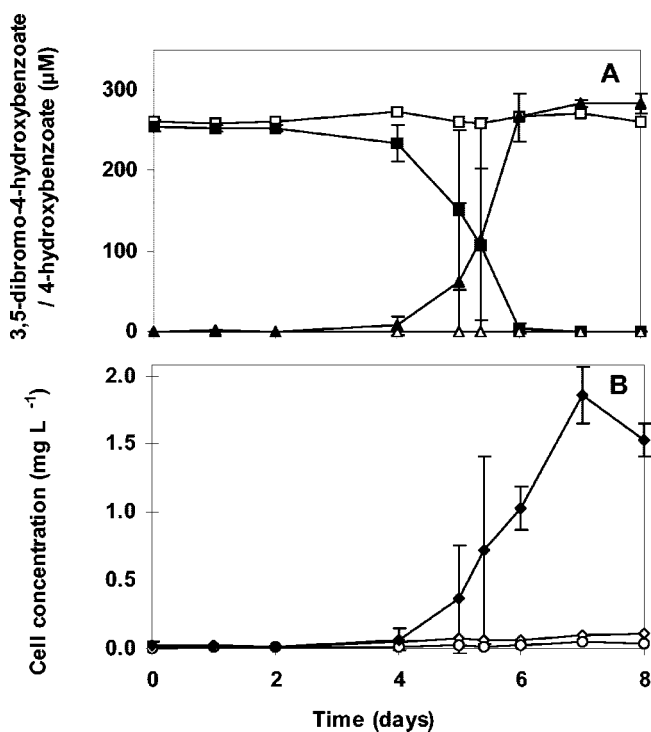


FIG. 3. DBHB removal in triplicate cultures (filled squares) and medium control (open squares), along with 4-hydroxybenzoate formation in triplicate cultures (filled triangles) and medium control (open triangles) (A); increase in biomass concentration ( $\text{mg liter}^{-1}$ ) determined by  $^{14}\text{C}$  incorporation to new cells (filled diamonds) (B). Points and error bars represent means and standard deviations for triplicate cultures. No growth was observed when either cells (open circles) or DBHB (open diamonds) was omitted.

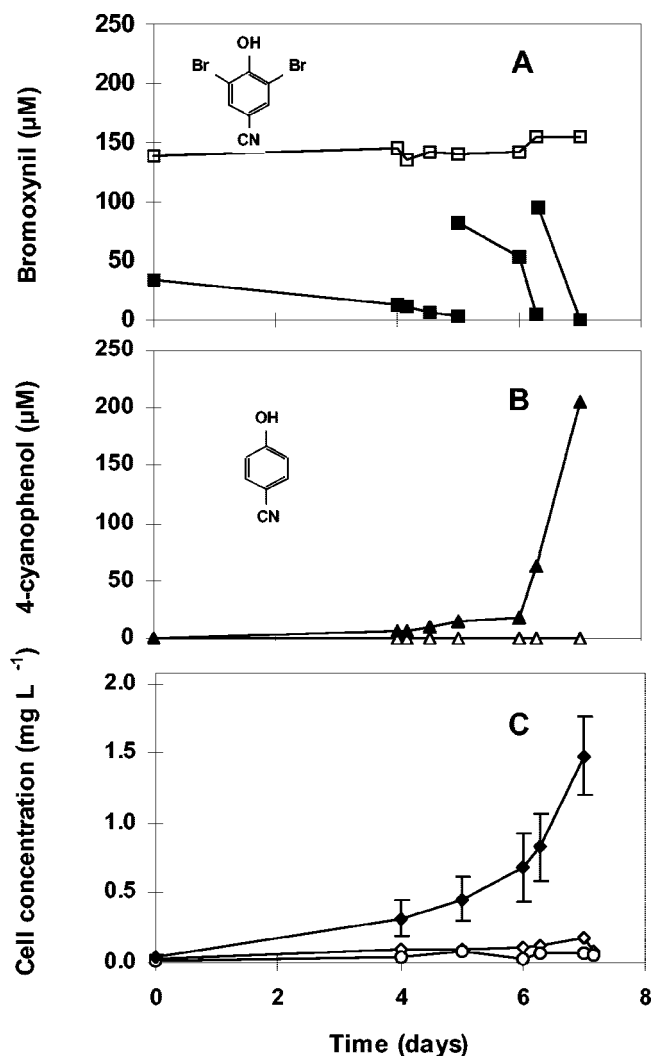


FIG. 4. Bromoxynil removal in cultures (filled squares) and medium control (open squares) (A), along with 4-cyanophenol formation in cultures (filled triangles) and medium control (open triangles) (B). Triplicate cultures illustrated similar trends (data not shown). Increase in biomass concentration ( $\text{mg liter}^{-1}$ ) was determined by  $^{14}\text{C}$  incorporation to new cells (filled diamonds) (C). Points and error bars represent means and standard deviations for triplicate cultures. No growth was observed when either cells (open circles) or bromoxynil (open diamonds) was omitted.

the final mass of the debrominated product (4-cyanophenol or 4-hydroxybenzoate) along with the final cell biomass, we calculated  $f_e$  and  $f_s$  to be  $0.78 \pm 0.03$  and  $0.22 \pm 0.03$  for growth on DBHB and  $0.74 \pm 0.02$  and  $0.26 \pm 0.02$  for growth on bromoxynil. These values are consistent with those previously reported (0.67 and 0.29) for *D. chlororespirans* during metabolic reductive dechlorination (38).

Debromination stoichiometry was investigated by plotting the parent depletion data against the metabolite formation data for both DBHB and bromoxynil (data not shown). Complete conversion of brominated parent to debrominated metabolite, without further degradation of metabolite, would theoretically result in a regression line with a slope of 1. The regression analyses determined mean slopes (with standard deviations for triplicate samples) of  $1.03 \pm 0.01$  ( $r^2 = 0.99$ ) and

$0.91 \pm 0.21$  ( $r^2 = 0.95$  to  $0.99$ ) for DBHB and bromoxynil, respectively, indicating debromination was indeed stoichiometric.

**Ioxynil deiodination.** No deiodination occurred when only ioxynil was present or when the cultures were amended with bromoxynil (samples were monitored for 36 days [data not shown]). The higher concentration of ioxynil ( $55 \mu\text{M}$ ) added at day 36 was also not transformed (samples were monitored until day 60 [data not shown]). In contrast, ioxynil was depleted when ClOHB was provided (ClOHB was also dehalogenated) (Fig. 5) with stoichiometric conversion to 4-cyanophenol (mass spectral identification of 4-cyanophenol [data not shown]). However, when ClOHB was added to the cultures previously amended with ioxynil and bromoxynil, no deiodination, debromination, or dechlorination was observed by day 60, suggesting a possible inhibitory interaction between the two herbicides.

## DISCUSSION

Here, we report the anaerobic transformation of bromoxynil by *D. chlororespirans*. We identify the transformation mechanism (reductive dehalogenation) and demonstrate that *D. chlororespirans* uses bromoxynil as an electron acceptor for growth. Additionally, we show that the aerobic bromoxynil metabolite DBHB is subject to the same transformation process, suggesting that *D. chlororespirans* could promote debromination in soils subject to anaerobic conditions and transient aerobic conditions. Previous research has focused primarily on aerobic bromoxynil degradation (15, 27, 43, 45, 48), with little information addressing bromoxynil's fate under anaerobic conditions. To our knowledge, there has been only one report (22) specifically addressing anaerobic bromoxynil degradation. In that investigation, the microorganisms responsible were not identified. Our study represents the first pure culture identified with the ability to anaerobically degrade bromoxynil.

Although biological debromination has been previously documented (1, 2, 3, 6, 10, 18, 30, 36, 38), there is a lack of quantitative information on growth rates when a brominated compound is used as an electron acceptor. In this study, we document growth rates on two brominated compounds ( $18.4 \pm 5.2 \text{ h}$  and  $11.9 \pm 1.4 \text{ h}$ ) indicating that metabolic reductive debromination has the potential to be as effective as metabolic reductive dechlorination for the sustainable remediation of halogenated contaminants. Thermodynamically, the reduction of brominated benzoates provides  $\Delta G^\circ$  values ranging from  $-146.5$  to  $-159.7 \text{ kJ per reaction}$  (12); therefore, it seems likely that sufficient free energy can be obtained from DBHB reduction. Based on the similarity in structure between DBHB and bromoxynil, along with the growth results presented here, bromoxynil reduction is also a thermodynamically favorable process.

The *D. chlororespirans* deiodinating transformation reported here is the first dehalogenating reaction to be documented for ioxynil. Studies investigating ioxynil's fate include the aerobic transformation of ioxynil to 3,5-diiodo-4-hydroxybenzamide by *Agrobacterium radiobacter* 8/4 (47, 49) and a report identifying seven ioxynil metabolites during groundwater recharge and bank infiltration (16). All seven ioxynil metabolites were halogenated, with two retaining the iodine groups and the others exchanging the iodine groups for other halogens. In our exper-



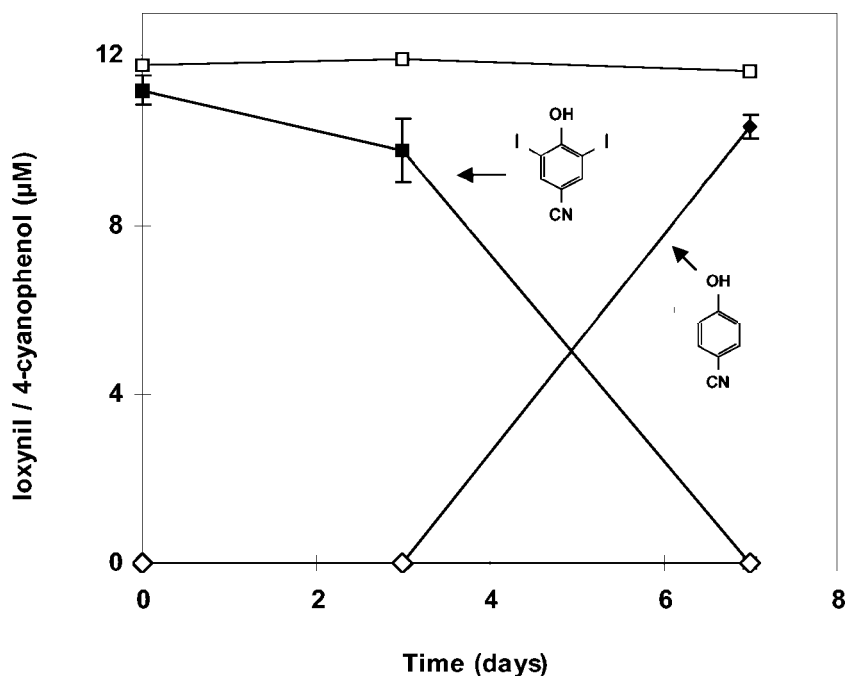


FIG. 5. Ioxnyl removal (filled squares) and 4-cyanophenol formation (filled diamonds) in cultures amended with ClOHB (1 mM). For the culture samples, points and bars represent means and standard deviations from triplicate cultures, respectively. The medium control is represented by open squares.

iment ioxnyl was completely deiodinated; however, unlike bromoxnyl, it cannot act as an electron acceptor for growth and was depleted only when ClOHB was supplied. This indicates that ioxnyl is more recalcitrant than bromoxnyl for *D. chlororespirans* and possibly more generally resistant to anaerobic degradation in soils. This potential inhibition of bromoxnyl degradation by ioxnyl has implications for agricultural sites because these herbicides are sometimes applied as a mixture.

Thermodynamically, the limited ability of *D. chlororespirans* to deiodinate ioxnyl is unexpected because the amount of energy released ( $-\Delta G$ ) increases with the increasing halogen atomic number (fluoro < chloro < bromo < iodo) (13). We can only hypothesize that the larger size of the iodinated compound inhibits its susceptibility to dehalogenation; for example, movement into the active site of the dehalogenase enzyme may be restricted. It is possible, however, that other soil microorganisms would be able to directly metabolize ioxnyl anaerobically via reductive dehalogenation. This requires further investigation.

Anaerobic deiodinating activity has been reported for enrichment cultures with 4-iodobenzoate (19) and with 2-iodophenol (29) and for one pure culture, *Desulfomonile tiedjei* (11). Recently, biological deiodination has received attention because triiodinated benzenes, used as X-ray contrast agents, have been found in sewage treatment plant effluent, surface water, groundwater, and raw drinking water (33, 44). Researchers illustrated the microbial dehalogenation of 5-amino-2,4,6-triiodoisophthalic acid (ATIA), an X-ray contrast agent precursor, in an anaerobic fixed-bed reactor (24) and in an enriched mixed culture (25). Based on 16S rRNA sequences from the reactor, four phylotypes were identified, with three belonging to the genus *Desulfitobacterium* and the fourth being

associated with the *Bacteroidetes* phylum (24). The authors suggest that the *Desulfitobacterium* species were probably responsible for ATIA deiodination, although no direct proof was provided. Our research provides direct evidence that a bacterium in the *Desulfitobacterium* genus indeed has deiodinating ability.

The current research documents the metabolic reductive dehalogenation of a brominated herbicide and metabolite by *D. chlororespirans*. In addition, the dehalogenation of an iodinated herbicide in, presumably, a cometabolic fashion is illustrated. This is the first direct report of deiodination by a bacterium in the *Desulfitobacterium* genus and the first report of an anaerobic pure culture able to transform bromoxnyl or ioxnyl. Since *D. chlororespirans* is a soil isolate, it is likely that it or a closely related strain could dehalogenate bromoxnyl or ioxnyl in a soil environment. Additionally, *D. chlororespirans* can form spores; thus, its survival under unfavorable (e.g., aerobic) conditions in soil is likely. This type of bacterium, along with other dehalogenating soil microbes living in anoxic microhabitats, is poised to respond to transient anaerobic conditions (e.g., when soils flood) and metabolize halogenated herbicides like bromoxnyl (4, 7, 14, 39). Future research will focus on actual contributions of anaerobic dehalogenation activity to the metabolic fate of halogenated pesticides in the soil environment.

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Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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